Project Report: Directed Evolution of an RNA Polymerase Ribozyme that Utilizes Highly Activated Monomers

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Project Progress

The goal of this proposal is the directed evolution from random pools of RNA of a polymerase ribozyme that utilizes highly activated nucleotide monomers as substrates. The successful identification of a ribozyme with these properties will represent a step towards the evolution of a self–replicating RNA and will provide insights into how simple replicating systems could have lead to the emergence of life on earth. This work also represents a potential advance in the synthesis of artificial life. Such a system would require replicable genetic information and a lipid vesicle to separate it from its external environment. Using highly activated nucleotide monomers as substrates for polymerization is advantageous in that they are less polar than nucleotide triphosphates (NTP), thus facilitating their diffusion through lipid vesicles. Furthermore, highly activated nucleotide monomers such as 5'–phosphorimidazolides have a 500–fold greater ability to polymerize on a template than NTP. Using these substrates will therefore reduce the rate enhancement required of the ribozyme to efficiently polymerize the monomers, which should simplify it.

The initial round of selection for the polymerase ribozyme requires a great initial expenditure of resources, the final preparations for which have occurred in the last 6 months. These preparations have included large–scale production of a random RNA pool from which the polymerase ribozyme may be selected, and smaller synthetic RNA molecules used in the selection scheme. Biological enzymes that will be used in the selection, including T4 DNA ligase and MMLV reverse transcriptase, were also produced. In addition, many small–scale pilot experiments were performed in order to ensure that the selection will yield the functional RNA molecules we desire. To date, all pilot experiments have shown positive results and the first round of selection for the RNA polymerase is scheduled to begin within 1 month of this report.

Highlights

- Pilot experiments have been performed to validate the ability of the selection scheme to enrich for RNA molecules with the ability to synthesize new RNA from nucleotide monomers.

- All materials necessary to begin selection of the polymerase ribozyme have been prepared.
- Round 1 of the RNA polymerase ribozyme selection is ready to begin.

Roadmap Objectives

- ***Objective No. 3.2:*** Origins and evolution of functional biomolecules